UNCLASSIFIED

AD NUMBER

AD437967

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; APR 1964. Other requests shall be referred to Army Biological Laboratories, Fort Detrick, Frederick, MD 21701.

AUTHORITY

BDRL, D/A ltr, 27 Sep 1981

THIS PAGE IS UNCLASSIFIED

UNCLASSIFIED

AD 437967

DEFENSE DOCUMENTATION CENTER

FOR

SCIENTIFIC AND TECHNICAL INFORMATION

CAMERON STATION, ALEXANDRIA, VIRGINIA

UNCLASSIFIED

NOTICE: When government or other drawings, specifications or other data are used for any purpose other than in connection with a definitely related government procurement operation, the U. S. Government thereby incurs no responsibility, nor any obligation whatsoever; and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use or sell any patented invention that may in any way be related thereto.

437967

437967

TECHNICAL MANUSCRIPT 132

RIFT VALLEY FEVER VIRUS ASSAY IN SERUM-FREE HAMSTER KIDNEY MONOLAYERS

UNCLASSIFIED BY DDC
AS AD NO. 437967

APRIL 1964

1964

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

NO OTS

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 132

RIFT VALLEY FEVER VIRUS ASSAY IN SERUM-FREE HAMSTER KIDNEY MONOLAYERS

John J. Boyle

Virus and Rickettsia Division
DIRECTOR OF BIOLOGICAL RESEARCH

Project 1C522301A05905

April 1964

The information in this document has not been cleared for release to the public.

DDC AVAILABILITY NOTICE

Qualified requestors may obtain copies of this document from DDC.

Foreign announcement and dissemination of this document by DDC is limited.

ABSTRACT

High titers of some arboviruses, including Rift Valley fever (RVF) virus, are obtained in cell cultures without any observable cytopathogenic changes. In the absence of serum, however, a rapid destruction of tissue-culture cells occurred with RVF virus. Preparation of virus dilutions in a serum-free maintenance medium and observation of infected tube monolayers has been found to be an effective method for achieving reproducible titrations of RVF virus. It is possible to calculate a median cytopathogenic effect dose ($CPED_{50}$) comparable to titers determined by a plaque technique but more sensitive than *in vivo* titration. The tube monolayer titration is more economical, more rapid, and simpler to perform than previously used methods.

THIS
PAGE
IS
MISSING
IN
ORIGINAL
DOCUMENT

TITRATION OF RIFT VALLEY FEVER VIRUS IN HAMSTER KIDNEY CELLS IN THE ABSENCE OF SERUM

Multiplication of certain arboviruses in cell cultures may not be accompanied by cytopathogenic changes (cpe) even though high titers may be obtained in extracellular fluids.[1] High titers of Rift Valley fever (RVF) virus also are obtained in various tissue-culture systems in the absence of any cellular changes. Experiments in this laboratory have shown, however, that a rapid destruction of tissue-culture cells by RVF virus occurred when serum was omitted from the medium. This observation suggested a more economical method for the titration of RVF virus than the plaque technique or the mouse titrations presently employed.

The data reported here were obtained with a third tissue-culture passage of a seed of infected-lamb serum of the van Wyk strain of RVF virus.[2] The hamster kidney (HK) cells used in these studies were propagated in a growth medium consisting of medium 199[3] plus 10% calf serum. Culture tubes (16 by 150 mm) were inoculated with one milliliter of cell suspension (500,000 cells per milliliter) and after a 24-hour incubation at 37°C, a confluent sheet of cells was obtained and the growth medium was removed. The cells were inoculated with virus suspended in serum-free maintenance medium and absorption was allowed to proceed for one hour. After the absorption period, fresh maintenance medium (serum-free) was added and the cultures were incubated at 37°C during the observation period.

The maintenance medium was prepared from a concentrated (10X) stock medium 199 diluted to 1X with distilled, demineralized[5] water. The 1X solution was further diluted with one volume of balanced salts solution (BSS) to make a final dilution of 1:2. Virus dilutions were prepared in this serum-free maintenance medium and 0.1 ml of each dilution was inoculated into each of four tube cultures. Although the dilutions of virus for the tube titrations were made in the maintenance medium, the growth medium containing calf serum had to be employed for the corresponding plaque titrations, because comparable titers of virus were not obtained when calf serum was deleted from the agar overlay. Futhermore, the use of serum-free maintenance medium in the plaque technique resulted in a decrease in the number and size of the plaques.

The endpoints of the titrations, based on the cpe, were calculated by the method of Reed and Muench[4] and expressed as a median cytopathogenic effect dose ($CPED_{50}$). Observations for cpe were made daily for ten days; however, final endpoints were usually achieved by the fourth or fifth days. For routine titrations, daily examination of tubes would not be necessary.

$CPED_{50}$ titers of RVF virus were directly compared with titers obtained by three other methods of titration: a plaque technique* and mouse inoculation by either intracerebral or intraperitoneal routes (Table I). The $CPED_{50}$ endpoints of the serum-free tube tests compare favorably with results obtained with the plaque technique. This method of titration appears to be as reproducible as the latter but more sensitive than the *in vivo* titrations. Examinations of the extracellular fluids were negative for virus even in cultures showing extensive cell damage. This may be partially due to the thermal lability of RVF virus in the absence of serum. Although further experiments are required to determine the underlying cause of cell destruction in the absence of serum, it is presently known that the effect is not seen with UV- or heat-inactivated virus, and the cpe can be prevented by specific immune serum.

This method of titration has many advantages over the plaque and *in vivo* techniques. It is considerably more economical of materials, simpler to perform, and more rapid. These results warrant additional investigations on tube titration in a serum-free medium with other arboviruses that can multiply in cell cultures without producing obvious cell changes.

TABLE I. TITERS OF RIFT VALLEY FEVER VIRUS DETERMINED BY VARIOUS TITRATION PROCEDURES

Virus Sample No.	Median Cytopathogenic Effect Dose, $CPED_{50}$	Plaque-Forming Units, PFU	Intracerebral	Intraperitoneal
1	8.3[a]	8.5	7.1	6.9
2	7.7	7.8	6.5	6.3
3	8.3	8.0	-[b]	-
4	8.0	8.6	-	-
5	8.0	8.0	7.0	6.0
6	8.5	8.2	7.5	7.0

a. Log_{10} per milliliter.
b. Not titrated.

* J. L. Runnels, unpublished data.

LITERATURE CITED

1. McAllister, R.M. "Viral encephalitides," Annual Rev. Med. 13:389-410, 1962.

2. Kashula, V.R. "The propagation and modification of strains of Rift Valley fever viruses in embryonated eggs and their use as immunizing agents for domestic ruminants," Univ. of Pretoria Thesis, 1953.

3. Morgan, J.F.; Morton, H.J.; and Parker, R.C. "Nutrition of animal cells in tissue culture. I. Studies on synthetic medium," Proc. Soc. Exptl. Biol. Med. 73:1-8, 1950.

4. Reed, L.J., and Muench, H. "A simple method of estimating fifty per cent endpoints," Amer. Jour. Hyg. 27:493-497, 1938.